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Challenges and perspectives in continuous glucose monitoring

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Diabetes is a global epidemic that threatens the health and well-being of hundreds of millions of people. The first step in patient treatment is to monitor glucose levels. Currently this is most commonly done using enzymatic strips. This approach suffers from several limitations, namely it requires a blood sample and is therefore invasive, the quality and the stability of the enzymatic strips vary widely, and the patient is burdened by performing the measurement themselves. This results in dangerous fluctuations in glucose levels often going undetected. There is currently intense research towards new approaches in glucose detection that would enable non-invasive continuous glucose monitoring (CGM). In this review, we explore the state-of-the-art in glucose detection technologies. In particular, we focus on the physical mechanisms behind different approaches, and how these influence and determine the accuracy and reliability of glucose detection. We begin by reviewing the basic physical and chemical properties of the glucose molecule. Although these play a central role in detection, especially the anomeric ratio, they are surprisingly often overlooked in the literature. We then review state-of-the art and emerging detection methods. Finally, we survey the current market for glucometers. Recent results show that past challenges in glucose detection are now being overcome, thereby enabling the development of smart wearable devices for non-invasive continuous glucose monitoring. These new directions in glucose detection have enormous potential to improve the quality of life of millions of diabetics, as well as offer insight into the development, treatment and even prevention of the disease.

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Introduction

Diabetes Mellitus is a global epidemic which affects almost 1 in 10 people.[1,2] The World Health Organization (WHO) expects

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that more than 1/2 billion adults will suffer from diabetes within the next decade.[2] According to the International Diabetes Federation (IDF), more people have died in recent years from diabetes-related complications (5mio) than from HIV/AIDS (1.5mio), tuberculosis (1.5mio) and Malaria (0.6mio) combined.[1] The disease is categorized into two groups. In diabetes type I, the body does not produce insulin and is unable to regulate glucose levels without external intervention. In diabetes type II the body is resistant to insulin, and does not properly control and maintain healthy glucose levels. In both cases, the risks of hypo- (low glucose levels) and hyperglycaemic (high glucose) levels are dangerous and can result in serious health problems and premature death. Acute hypoglycaemia can cause patients to fall into coma, can induce seizures and result in death. Chronic hyperglycaemia results in organ, nerve, cardiovascular, and retinal damage.[3–5]

The first and most important step in treating diabetes patients is maintaining healthy glucose levels. This requires continuous, accurate monitoring of glucose levels over the day. Healthy blood glucose levels are very constant: between 4–7 mM,[6] or 70–120 mg dL^{-1}, while diabetic glucose levels fluctuate widely, between 2–30 mM[7] or 35–550 mg L^{-1}. Methods for accurate and reliable glucose detection are crucial for correctly and effectively diagnosing hyper and hypoglycaemia.

Conventional methods for glucose detection are biochemical. A blood sample is provided on a strip or substrate containing oxidizing enzymes. The reaction flux is then monitored by measuring either electrical or optical changes in the enzymatic substrate. This approach requires blood samples, and is therefore invasive. Further, the patients are burdened with the responsibility of monitoring their own glucose levels, and most patients need to perform intermittent measurements throughout the day. Critical fluctuations in the glucose levels may therefore go unnoticed either due to low patient compliance or unexpected side effects related to factors such as changes in insulin treatment, unknown food ingredients, and variations in hormone levels.[4]

Further, enzymatic detection of glucose has limited accuracy. The most common errors in detection are related to the quality of the enzymatic strips and patient error. Most strips can be stored for two years under ideal conditions, however the lifetime is significantly reduced by environmental stress, such as elevated temperature and oxygen (*e.g.* changes in altitude). Low quality strips may additionally not have complete enzyme coverage. Simple errors, such as testing with unwashed hands, will further reduce the accuracy of the measurement.[4,8–10] Many patients are not aware that the measurement requires that a specified volume of blood is deposited on the strip in order to convert the measurement signal to a glucose concentration. Glucose meters do not register sample volume, therefore variations in sample volume may lead to errors in the calculation of glucose concentration.[11] In addition, the enzymatic strips are selective for specific glucose conformations. Factors such as temperature and pH influence the equilibrium ratio of these molecular conformations. While the temperature and pH of blood is relatively constant, the slow interconversion between molecular conformations of the glucose molecule may be very relevant for glucose detection in other body fluids, such as sweat. In this case, fluctuations in temperature can result in non-equilibrium conditions. Lastly, using enzymes means that the strips can only be used once, or for a short period of time. As many diabetics will use anywhere between 5–20 strips each and every day, this leads to accumulated cost and waste.

New approaches for glucose detection that target the physical rather than the chemical properties of the molecule may overcome these current limitations. Ideally glucose detection could be performed not in blood, but in the interstitial fluid that surrounds the fragile nerve cells. Current research efforts in the field are focused on glucose detection through the skin, or in bodily fluids such as sweat, tears and saliva. To do this, new technologies with increased detection sensitivity and reliability are needed.

Non-invasive methods to detect glucose would enable a new paradigm in glucose monitoring. Patients could follow the progression of their glucose levels over time, allowing them to detect and quickly treat extreme fluctuations in glucose levels. Continuous glucose monitoring would vastly improve the quality of life for diabetes patients as well as aid in the understanding of diabetes development and prevention.[3,12–15] Continuous glucose monitors would also be very helpful for dieticians to find out individual responses to food, as this can strongly differ.[16]

The topics of glucose sensing and continuous glucose monitors have been covered in excellent reviews. Bruen *et al.* (2017) review the most recent advances in glucose sensors for different bodily fluids.[6] Wang *et al.* (2015) reviewed recent advances in electrochemical sensing.[3] Lin *et al.* (2017) focussed on key challenges and recent advances in the field and provide an extensive focus on newcomers in the market.[17] McCaul *et al.* (2017) survey several technologies and the accompanying challenges and opportunities with specific focus on sweat analysis.[18] Kim *et al.* (2018) reviewed the recent advances for non-invasive technologies using interstitial fluid and sweat.[19] Oliver *et al.* (2009) review and explain the background behind technologies used for glucose sensing.[20] The different factors analysed in these review articles highlight the multidisciplinary approach necessary for tackling the problems.

This review focusses on the physical mechanisms of glucose sensing, with the aim of identifying approaches that provide the required sensitivity and reliability to enable continuous, non-invasive glucose monitoring. For this, a detailed understanding of glucose concentrations, and the time lag with respect to blood, as well as fluctuations in pH and temperature in different body fluids is needed. Glucose concentrations in blood, interstitial fluid, sweat and tears, are correlated. Blood has the highest glucose levels, and the continuous decrease in glucose concentration from blood to the skin presents a bottleneck for non-invasive glucose detection. We discuss the glucose concentrations in these fluids, and the experimental work on correlating their values. This provides a guideline for the required sensitivity of glucose detection with non-invasive approaches. In order to understand the full scope of the current research, the review will start with a closer look at the molecular

properties of glucose. Strangely, this is often overlooked in the literature, although the complex properties of the glucose molecule are critically important when developing new approaches in glucose detection. The conventional enzymatic approach for glucose detection is described, along with detection limitations. We then review recent non-enzymatic approaches with examples and future prospects. Finally we survey the field of commercially available as well as emerging glucose detection technologies. Notably, the current market consists entirely of enzymatic glucose detectors, with a single exception (Eversense Senseonics). This demonstrates the urgency of addressing the potential and limitations of current approaches in non-invasive glucose detection, and the need for more research in this direction. We note that some state-of-the-art technologies will be missing from this review because the market is very competitive and thus patents are pending and innovations are not always published. While many non-invasive approaches for glucose detection are being researched, there are still many hurdles to overcome for commercialization. Finally, an overview will be given on the technologies in the current market for glucose monitoring.

Standards for accuracy in glucose detection

Measuring glucose accurately is the first step in diabetes patients' wellbeing and care. The accuracy of self-monitoring of blood glucose determines how well the patients are able to monitor and react to fluctuations in their glucose levels. *Accuracy* is how close the mean value is to the true concentration, while *precision* is how much measurement points vary with respect to one another.

Since 2013 the International Organisation of Standardization (ISO) requires glucose meters to provide measurements within a 15% margin of the real concentration.[21] The chart below shows this comparative accuracy of a glucometer (Fig. 1). 95% of the data must be in zone A, while 99% must be in zones A and B. In the glucose sensing community, accuracy is commonly defined in terms of the Mean Absolute Relative Difference (MARD). MARD is a way of defining how accurate the detection method with respect to another (accurate) system, usually the well-established Yellow Springs Instrument (YSI) 2300 chemical analyser. There are, however, no standardized ways to determine the accuracy and precision of glucose meters. This means that this is not necessarily a barrier for glucometers to enter the market.[9] Besides, sensors produced before 2013 do not necessarily meet the new, stricter guidelines.

Several comparison studies on commercial glucometers have revealed that many commercial self-testing devices do not meet the required standards when subjected to laboratory testing conditions. According to Freckmann *et al.* (2012) 80% of the most popular commercially available glucometers provide readings within the (pre 2013) ISO limits of a 20% margin.[9] Only half the glucometers measured provided readings within a 10% range of the true concentration (shown as relative bias% in Fig. 2). In 2016 another comparison was published where it was shown

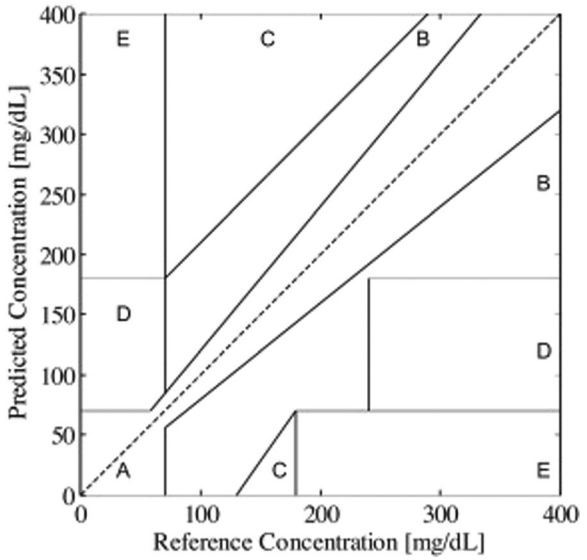

Fig. 1 Clarke's error grid analysis shows the accuracy zones for glucose detection. The *y*-axis depicts the measured data from the glucometer, and the *x*-axis depicts data from a reference biochemical analyzer (usually YSI 2300). Reprinted from ref. 22 with permission from Elsevier.

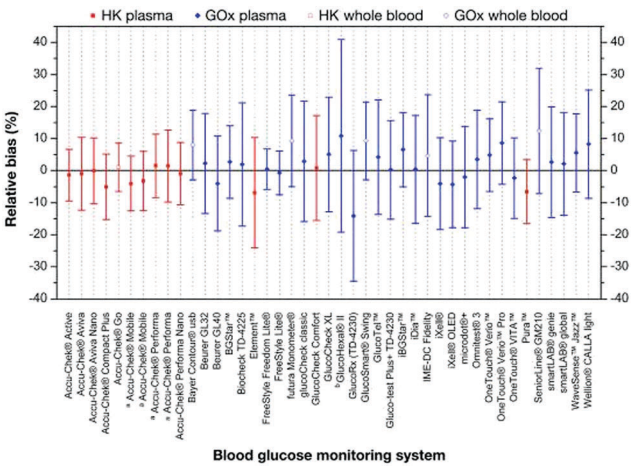

Fig. 2 Comparison of the accuracy of different commercially available glucometers depicted in terms of relative bias (%). From Freckmann *et al.* used with permission of the publisher, SAGE Publications, Inc.[9]

that none of the (anonymously) tested glucometers were within the 10% margin proposed by the FDA.[23] Ekhlaspour *et al.* (2017) performed a recent comparative study, and showed that just 7 of the 17 tested glucometers (test-strip-based) met the ISO 2003 standards, while only 2 (!) met the ISO 2013 criteria (Table 1).[24] The MARD of these 17 glucometers ranged from 5.6% to 20.8%. The only continuous glucose monitor found to be within the required 10% limit was the DEXCOM G5.[13]

These comparative studies analyzed commercially available glucometers. The results highlight the challenges faced by emerging technologies for non-invasive glucose detection that target body fluids with lower glucose concentrations than blood. These new glucose sensors must be able to detect low glucose concentrations with accuracies that meet increasingly strict standards.

Table 1 Commercial test strip brands for glucose monitoring ranked by Mean Average Relative Difference (MARD). The columns indicate which brands met the ISO 2003 and 2013 standards for glucose detection (data adapted from Ekhlaspour *et al.* (2017))[24]

Brand	MARD (%)	ISO 2003	ISO 2013
Contour Next	5.6	Yes	Yes
StatStrip Xpress	6.3	Yes	Yes
OneTouch VerioIQ	7.1	Yes	No
Accu-Chek Nano	7.3	Yes	No
FreeStyle Freedom Lite	7.5	Yes	No
Accu-Chek Aviva Plus	7.6	Yes	No
FreeStyle Lite	8.2	Yes	No
Nova Max	9.7	No	No
TRUEresult	13.0	No	No
HemoCue Glucose 201	13.2	No	No
OneTouch Ultra2	13.6	No	No
ReliOn Prime	14.3	No	No
BREEZE® 2	15.8	No	No
ReliOn Micro	16.0	No	No
AgaMatrix PRESTO	16.2	No	No
AgaMatrix JAZZ	16.7	No	No
SideKick	20.8	No	No

It is challenging to strictly assess and compare the accuracy of different emerging technologies for glucose detection in the literature. While commercially available glucometers are evaluated according to international standards, emerging technologies are commonly characterized according to the smallest concentration of analyte that can be detected, the limit of detection (LOD).

The anomeric properties of glucose

Interestingly, in the field of glucose detection, the properties of the glucose molecule, and how these impact detection accuracy, are often overlooked. While the properties of blood are very stable, the pH, temperature, and composition of other body fluids fluctuate over time. This impacts the anomeric ratio of glucose molecules in the body fluid. For detection approaches that are anomer-specific, *e.g.* enzymes, this can lead to errors and unreliable readings, as the timescale for anomer equilibration (hours) is significantly longer than the time needed to perform the measurement (seconds). New approaches for non-invasive, continuous glucose sensing that target body fluids with more volatile properties than blood must account for these effects. In this section we review the anomeric properties of glucose, and the potential impact on glucose detection accuracy.

Glucose is a simple sugar and a primary source of energy in the human body, and one of the most abundant carbon-based molecules in nature. As with other sugars/carbohydrates, the structure and chemistry of glucose is complex. To explain the important differences between carbohydrates, one has to start with the term chirality. A chiral center is a carbon atom which has 4 different substituents (groups). Each chiral center has 1 of 2 different structures, called either *R* or *S*. An *R* or *S* structure can lead to significant differences between two molecules. Carbohydrates have several chiral centers, resulting in unique structures. Mannose, glucose, galactose, gulose, and altrose are examples of molecules that only differ from one another by chirality. These molecules are called stereoisomers, which means that they have the same chemical formula, the same chemical bonds, but different 3-dimensional configurations. Generally the number of stereoisomers a molecule has is given by 2^n, where n is the number of chiral centers. Glucose ($C_6H_{12}O_6$) contains five chiral centers, and belongs to the aldohexose group, composed of 16 stereoisomers. There are 16 stereoisomers and not 32, due to the anomeric carbon. Glucose itself forms two stereoisomers, L-glucose and D-glucose. The L and D form are mirror images of each other, *i.e.* enantiomers. Both L-glucose and D-glucose are chemically stable, but only D-glucose is found in nature. When we refer to glucose in this review, we are referring to D-glucose. The glucose molecule is also known as glucopyranose, dextrose, or its systematic name (2*R*,3*S*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexanal.

In aqueous solutions, one linear chain and two cyclic forms of glucose exist in a dynamic equilibrium. Over 99% of glucose molecules exist in one of the two cyclic six member (pyranose) forms, while less than 1% of glucose molecules are in the open chain configuration. The chain, γ-D-glucose, is not thermodynamically stable, but serves as an intermediate for conversion between the cyclic forms. The closing of γ-D-glucose can happen at 2 different angles, producing an anomer with either an *R* or *S* chiral center: α-D-glucose or β-D-glucose. The interconversion between α-D-glucose and β-D-glucose *via* the linear chain γ-D-glucose is shown in Fig. 3.

As only 1 chiral carbon differs, the subclass of stereoisomers are known as anomers (only applicable for sugars) or epimers.[25,26] The location of this chiral center is known as the anomeric carbon. The α and β anomers differ only in the orientation of one of the OH groups, but have different chemical properties. In α-D-glucose the OH-group is in the axial position at the anomeric carbon, while in β-D-glucose the OH-group is in the equatorial position (Fig. 3). In carbohydrate chemistry the general rule is that the axial position (α-D-glucose) is more stable than predicted by theory, known as the *anomeric effect*. Two major factors influence the preferential position of the substituent at the anomeric

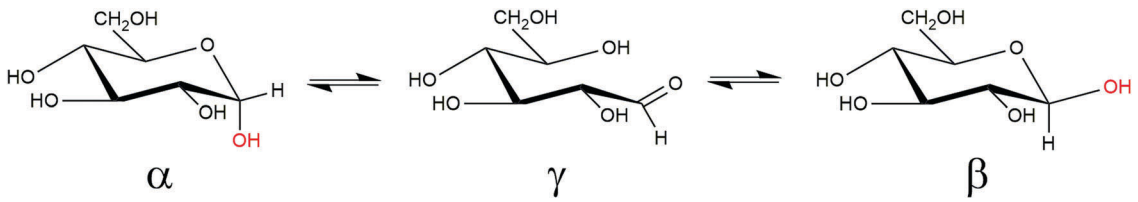

Fig. 3 Schematic of the mutarotation of D-glucose. The interconversion between the α-D-glucose and β-D-glucose ring forms occurs *via* the linear γ-D-glucose form.

carbon: polarity of the solvent and the substituent groups.[27] Water is highly polar and glucose has relatively small groups, and glucose in physiological conditions is (unusually) more prevalent in the equatorial position (β-D-glucose) over the axial position (α-D-glucose).

In the chair conformation the OH group at the anomeric centre of the β-anomer is in the equatorial position, which is more stable.[25,28] It points away from the molecule resulting in less steric hindrance and less electric repulsion.

The precise mechanism of the interconversion of glucose anomers is not yet fully understood, and thus it is the focus of extensive experimental and theoretical research.[28–33] Fig. 4 shows a schematic of the energy diagram, including the activation energy E_A required for interconversion between the cyclic and linear forms of glucose. Depending on the properties of the solvent, the α-D-glucose or β-D-glucose form may be more favorable. Intra- and intermolecular hydrogen bonds (mainly with water) play a large role in the difference in stability between the anomers.[30,32] Further the ratio of α to β glucose anomers depends on temperature and pH, as well as concentration, and solute.[7,31,34] Basic conditions prefer β-D-glucose, and acidic conditions prefer α-D-glucose.[34] Interconversion between the anomers is very slow; it takes hours for the equilibrium to be reached.

Generally the glucose molecule is very stable, and therefore difficult to detect chemically. However, α-D-glucose and β-D-glucose can be distinguished from one another by some enzymes, and they have different functions in the body.[35,36] Some enzymes can react with both anomers, for example hexokinase (the starting enzyme for glycolysis) and glucokinase, albeit with some selectivity for the β anomer.[7,37,38] Due to the selectivity of enzymes, humans can digest both α and β glucose (with some preference for β), but can only digest polymers of α-D-glucose. The difference between α-D-glucose polymers and β-D-glucose polymers is striking: polymers of α-D-glucose are starches, whereas polymers of β-D-glucose are cellulose (in addition, each following glucose molecule is inverted in the chain). Other enzymes, including the most popular enzyme for glucose detection, glucose oxidase (GOx), is specific for β-D-glucose, therefore the pH of the sample must be known for accurate glucose readings.[7,37,39] The temperature and pH of blood are very constant, and the ratio of β : α glucose is assumed steady at a similar ratio as in water, 64 : 36.[7,25] Total glucose concentrations in blood samples can therefore be calculated with a glucose meter based on the concentration of oxidised β-glucose.

The anomeric effect can pose a serious challenge for the detection of glucose[40] in body fluids other than blood. For example, if the pH drops to 5.5 for over an hour, the ratio of the anomers have shifted, but not yet reached equilibrium. This means that glucose detection with anomeric-selective enzymes, such as GOx, may not yield reliable results.

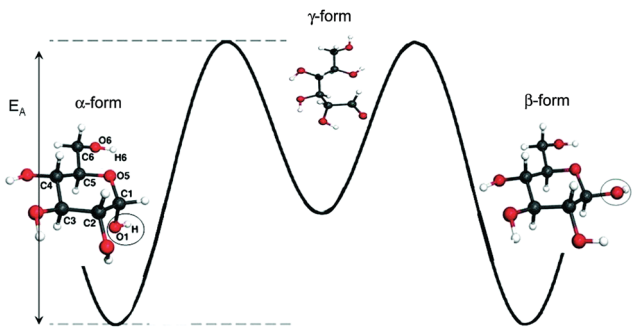

Fig. 4 Energy diagram of the 3 forms of D-glucose. The rate of interconversion between glucose forms is determined by the activation energy E_A. Reprinted with permission from Dujardin *et al.*[29] Copyright (2011) American Chemical Society.

Glucose concentrations in different bodily fluids

Traditionally urine was used to establish whether a patient was diabetic, as in contrast to healthy patients, the urine of untreated diabetics with hyperglycaemia contains glucose. For the treatment of patients, however, blood is the most common body fluid to use for glucose detection. Blood is relatively easy to obtain and the measurement of blood glucose levels is well established. Moreover, glucose concentrations in blood are relatively high, and therefore enable good detection accuracy and reliability. Further the temperature, pH and composition of blood are very constant, which improves detection accuracy and reliability. However the extraction of blood samples is invasive and uncomfortable. Approaches towards non-invasive glucose detection have targeted other body fluids such as interstitial fluid,[15,41,42] sweat,[43–46] saliva,[47] tears[14] and even breath.[6,7,14,43,48–50]

The challenge in establishing less invasive glucose testing on these body fluids is that the glucose concentrations are lower, while the composition of the body fluid is variable. This means that non-invasive technologies must be able to reliably detect low concentrations of glucose in fluids with varying pH, temperature, and time delay (time lag) in glucose concentrations (defined in comparison to blood glucose levels). This may require additional sensors and/or frequent calibration of the glucose sensor to ensure reliable readings.

Table 2 summarizes the different body fluids used for glucose detection, as well as the advantages and disadvantages of using these fluids to monitor glucose levels. The importance of the pH value in glucose measurements should be noted. While blood pH is constant between 7.35–7.45, the pH of sweat can strongly vary due to the presence of lactic acid. The pH of interstitial fluid varies widely, as in contrast to blood, there are few buffering molecules to regulate pH. Due to the accumulation of lactic acid on the skin, the pH values of sweat is even more volatile. The time lag represents the time required for fluctuations in blood glucose levels to be detected in other body fluids.

Although promising results have been reported, measuring blood glucose levels non-invasively through the skin is problematic due to the heterogeneous distribution of tissue types.[52,53] A logical choice of fluid for minimally invasive to (ideally) non-invasive glucose measurements is interstitial fluid. Interstitial fluid is directly linked to the blood stream, with a time lag of

Table 2 Summary of the different body fluids used for glucose detection, the potential advantages and disadvantages of targeting these body fluids for glucose detection, and the time lag in changes in glucose concentration with respect to blood

Bodily fluid	Advantages	Disadvantages	Time lag (min)
Blood	Highest concentrations (2–40 mM) Relatively easy to extract Stable pH (7.35–7.45)	Invasive	—
Interstitial fluid	High concentrations (2–20 mM) Fluid surrounding nerve endings Some research is done measuring through the skin	pH varies from 6.6–7.6[5] Microneedles or implants are usually necessary	0–45 (avg. 8–10)[51]
Sweat	Easy sample collection Non-invasive	pH varies between 4.5–7 Low glucose concentrations Individual calibration necessary for reliable results[43,45,46]	20, but lags behind after interstitial fluid[48]
Ocular fluid (tear fluid)	Easily accessible	pH varies from 6.5–7.6 Mildly invasive Low volume[50] Tears from emotion and fro irritation differ in composition[50]	5–30[6,50]
Saliva	Easy sample collection	pH varies strongly from 5–8 Residual food and drink can falsify the reading Low glucose concentration Long time lag	15–20[50]

about 10 minutes. Though blood glucose levels will give earlier warnings, interstitial fluid may actually a more relevant measurement fluid for diabetics because it is closer to the nerve endings and damage occurs when the glucose levels of the interstitial fluid fluctuate.[51]

Using saliva for glucose measurements presents several challenges and not many advantages, as the composition varies widely and it may be inconvenient. Sweat monitoring is gaining popularity, especially because it is non-invasive and comfortable for patients, but individual calibration and a high pH variance means that more research is required to obtain reliable readings. In addition, it would even be an appreciated monitoring fluid for athletes. Athletes could follow the state of their body through glucose measurements as well as lactic acid measurements. Lactic acid is produced by overly exerting the muscles, and is thus an indication of muscle fatigue. Ocular fluid (tear fluid) is also promising, and glucometers may one day be easily inserted into contact lenses. One of the major advantages is that stability is not an issue due to the fact that contact lenses are replaced regularly anyways.

Enzymatic glucose sensors

Nearly every glucose sensor on the market does not measure glucose directly, but measures the reaction product arising from glucose molecules that react with an oxidizing enzyme. The most popularly used enzyme is glucose oxidase (GOx), followed by glucose dehydrogenase (GDH). The measurement is performed by puncturing the skin to retrieve a blood sample, which is then deposited on a substrate containing glucose oxidase. The volume of blood used for the reaction is controlled through the size of the sample chamber.[4]

The β-D-glucose anomer reacts with GOx to form gluconic acid. Gluconic acid then undergoes a further reaction, depending on the generation of biosensor. A calibrated glucometer measures the reaction flux to determine the glucose concentration. The glucometer detects this by monitoring changes in either the electronic or optical properties of the strip. The optical detection is usually colorimetric, based on the change of colour in the strip. The electrical detection is amperometric,[7] and records the change in conductivity of the strip.

The catalytic centre of the enzyme GOx is flavin adenine dinucleotide (FAD). The FAD is reduced to $FADH_2$ during the oxidation of glucose. To measure the glucose concentration, $FADH_2$ is oxidized back to FAD, and each generation of glucose biosensors does this in a different way (Fig. 5). The first generation uses hydrogen peroxide. The second generation uses a mediator such as ferricyanide. Finally, the third generation directly oxidizes $FADH_2$ through electron transfer from the electrode.

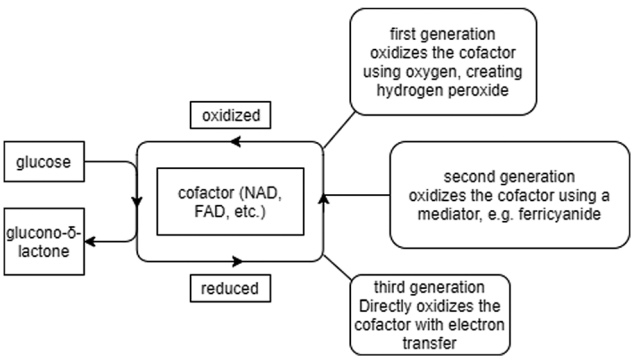

Fig. 5 Schematic depicting the three generations of enzymatic glucose sensors. Adapted from Murugayan *et al.*[55]

Glucose detection using GDH measures both α-D-glucose and β-D-glucose. Glucose detection using GOx only measures the β-D-glucose anomer, so the ratio of α-D-glucose and β-D-glucose needs to be known to calculate the total glucose concentration.[54] Thus, for enzymatic detection of glucose, the anomeric effect may need to be accounted for. Due to the stable pH and temperature in blood, the glucose anomeric ratios are assumed to remain constant, and it is straightforward to calculate glucose concentrations, even with detection approaches that are sensitive to only a single anomer. For bodily fluids which vary in pH and even temperature this is more difficult, especially if the pH fluctuates, as it takes time for the anomers to reach equilibrium.

The big disadvantage of enzymatic approaches is that the detection relies on one-way substrates that must be continuously replaced. Besides the accumulated cost and production of waste, this is a bottleneck for long term use of continuous glucose monitors. However, the current top-of-the-line commercially available enzymatic-based continuous glucose monitors last for up to two weeks and show promising results. Among others Abbott's FreeStyle Libre, Medtronics and DEXCOM's G5. More information on these commercially available continuous glucometers is presented in the chapter covering the current market.

Hospital laboratories and research facilities apply more sophisticated equipment for glucose detection than patients and general practitioners (GPs). One example is the popular YSI chemical analyser. These chemical analysers are large and expensive, but can detect a range of metabolites, including β-D-glucose, hydrogen peroxide, lactic acid, ethanol, glutamate, lactose, and sucrose, using a variety of specialized enzymatic sensors, all with high accuracy and precision (MARD of 2%). Typically, comprehensive biochemical analysis is too expensive for GPs and small medical practices. Smaller machines offer a less comprehensive metabolite analysis, for example, the Biosen analysers measure glucose and lactate in blood samples.

For more information on enzymatic sensors, readers are referred to the review by Heller and Feldman (2008) and by Ferri *et al.* (2011).[56,57]

Electrical and electrochemical approaches

Enzymatic detection can be combined with more sophisticated detection strategies to yield higher detection accuracy. For example electrical and electrochemical measurements combined with enzymatic detection can be performed on body fluids containing lower glucose concentrations than blood. Several research groups have made extraordinary advances in glucose monitoring using this approach. Below, some of these novel technologies are introduced.

Often, enzymes are mixed or bound to the electrode materials in order to increase sensitivity. Lee *et al.* (2017) made a flexible patch that can easily be attached to the skin and which monitors glucose concentrations in sweat.[45] The device measures glucose electrochemically using highly selective electrodes. The electrodes consist of porous gold to increase the electrode active area, and coated with GOx. A negatively charged nafion layer is used to immobilize the enzyme and simultaneously block diffusion of negatively charged ions which can falsify the measurement. A humidity sensor is used to detect when the critical sweat volume for measurement has been obtained. The sensor works for one day without calibration. The device even includes a drug feedback delivery system, using microneedles for treating diabetes patients. The long term stability of the GOx electrode remain an issue.

Similarly, a sweat-monitoring device was reported that measures a range of metabolites and properties of sweat in order to calibrate the glucose measurement: glucose, lactic acid, K, Na, pH, and temperature.[46,58] The glucose and lactate were both monitored with amperometric measurements. The glucose sensors work for just 2 hours before needing replacement. While the electrodes for electrolyte detection need to be calibrated, no calibration is needed for the glucose and lactic acid detection. The gold electrodes are coated with an electrochemically deposited Prussian Blue layer, followed by a layer of mixed polysaccharide chitosan/GOx/single-wall carbon nanotubes. As the potassium concentration in sweat is constant, this metabolite can be used as a measurement reference.[6] An important parameter for sensor design and calibration is that the glucose concentrations vary over the body, meaning that sweat from the armpit varies from lower back sweat.[59]

Zinc oxide (ZnO) electrodes functionalized with GOx were combined with impedance measurements to detect glucose, resulting in measurements. The results yielded readings within 15% of the values using TRUEresult glucometer, which has a reported 95% accuracy.[44] The LOD was reported to be 0.1 mg dL^{-1} (0.06 mM).

Zhang *et al.* (2018) also used ZnO (nanowires) in combination with GOx, but in their setup no external battery power is needed.[60] The device relies on electricity from the *piezo-enzymatic-reaction* coupling on the GOx/ZnO. Piezoelectric response is due to a potential from mechanical stress (in this case through muscle movements). The device can monitor glucose in real-time and was successfully tested on mice while it showed an LOD of 0.019 g L^{-1} (0.1 mM).

Yehezkeli *et al.* fabricated electrodes using GDH and gold nanoparticles.[61] These electrodes are oxygen insensitive due to the flavin dependency of the GDH. Their approach enabled detection of glucose concentrations as low as 60 mM.

Yao and Zhang fabricated a paper based electrochemical device using carbon-based electrodes and GOx, specifically for detection of uric acid and glucose in urine.[62] The limit of glucose detection was 0.35 mM.

A further innovative approach consisted of "tattooing" electrodes in the skin in order to extract interstitial fluid.[42] The interstitial fluid was collected by iontophoresis, and then analysed by an enzymatic amperometric system. A special gel was applied to prevent the issue of skin irritation from iontophoresis. Vega *et al.* (2017) has done a proof-of-concept study on tattoos in which GOx is in the ink, and the colour of the tattoo is related to the glucose concentration of the skin.[63]

Tear sensors also exist, as a contact lens with biosensors. Contact lenses are routinely replaced every day, so the sensors

do not need to be stable for a long period of time, and thus enzymatic sensors are a practical choice. The problems arising from the use of contact lenses are battery power due to lack of space and interference in glucose detection by other analytes.[6,50] Park *et al.* innovated on this concept by applying GOx in a soft contact lens combined with transparent nanomaterials.[64] Catalase (another enzyme) was included to break down hydrogen peroxide, thereby increasing the GOx sensitivity.

Liui *et al.* created a glucose sensor for tears and saliva using GOx mixed with DNAzyme.[65] The ''pistol-like'' DNAzyme (PLDz) performed a self-cleavage reaction when in contact with hydrogen peroxide. The LOD was 5 μM.

Contact-less detection can also be combined with enzymatic detection. The detection of glucose oxidation can be done with optical spectroscopy if a marker is used to colour the solution after the oxidization of glucose (in this case using GOx). This way, very low glucose concentrations can be measured.

Colorimetric measurements have the same basis as electrochemical measurements, but instead of the current being measured, the amount of reactant is measured through a change of colour. This change of colour is induced by a reaction between hydrogen peroxide and a peroxidase. Colorimetric measurements continues to demonstrate promising results. Recently, Kim *et al.* applied nanoceria (cerium oxide) for colorimetry to improve detection sensitivity.[66]

An optical technique that is promising – although still in the early stages – is a metal waveguide capillary (MWC) compact photometer for measuring glucose in nanomolar concentrations.[67] This detection method uses absorption and scattering to detect glucose. Usually such setups are quite large, which isn't practical for patient glucometers. Miniaturisation is an important aspect in setups where light refracts because the further away the sensor is, the easier it is to detect the different wavelengths. By using a reflective metal capillary between the sample and the sensor, light is confined, but the angle can still be measured and detection is enhanced 3000-fold compared with commercially available spectrophotometers, namely an LOD of 5.12 nM.

Non-enzymatic glucose sensors

The benefit of enzymatic sensors is that they are specific for glucose, thereby enabling good detection accuracy under controlled conditions. However enzymatic sensors are not stable, and therefore require regular replacement.

Non-enzymatic glucose sensors (NEGS) are the focus of intense research because they offer the major benefits of increased stability and lower maintenance,[49] the potential for continuous glucose monitoring without sensor replacement, and the possibility to detect glucose non-invasively.

NEGS can be roughly divided into two categories: sensors that combine artificial enzymes for the electrochemical detection of oxidized glucose, and sensors that physically detect the glucose molecule. Artificial enzymes combined with electrochemical detection is analogous to the enzymatic approach described in the last section, but specialized electrode surfaces are used instead of enzymes.[7,37,49,68] Some examples of these are given in the next subchapter. For a fuller review on this topic, readers are referred to reviews by Toghill and Compton (2010), Park *et al.* (2006), Wang (2015), and Zaidi and Shin (2016).[3,7,49,68]

Physical glucose detection, on the other hand, is based on detecting specific properties of the glucose molecule. This is commonly done with optical spectroscopy, dielectric and impedance spectroscopy, and electric measurements.[44,52,69,70] The optical approaches include vibrational spectroscopy, *i.e.* infrared (IR)[22,71,72] and Raman spectroscopy,[41,53,73–76] and fluorescence spectroscopy.[13,77]

One of the benefits of non-enzymatic glucose detection may lie in the potential to detect different glucose anomers, as well as differentiate between them. Vibrational spectroscopy can be used to clearly identify and distinguish glucose anomers, due to small differences in the spectra. The α-D-glucose and β-D-glucose anomers can be clearly distinguished in IR-spectra[78] and Raman spectra.[29,79,80]

In the case of approaches using artificial enzymes, it is more difficult to determine the impact of the anomeric effect. Not much research has been done in this direction, but there seems to be a preference for selective electrodes to oxidize α-D-glucose.[7] More detailed electrochemical analysis of glucose anomers is required, and electrode materials and surfaces should be assessed on the anomer selectivity for higher detection accuracy.

Stabilization of a glucose anomer on a functionalized substrate improves the accuracy of glucose detection immensely for all techniques. Dingari *et al.* (2012) used albumin on gold electrodes to stabilize the glucose anomers[75] for Raman detection. Similar results have been shown using bis-boronic acid and other molecules derived from boronic acid.[3,6,49,81] The glucose molecule does not interconvert between anomers once it is bound. The boronic acid derivatives have been shown to work for glucose detection in fluorescence, impedance, and Raman measurements.[3]

This approach has yielded promising results for more accurate glucose detection. However, a current limitation is that boronic acid is even more selective for fructose than for glucose.[49] Further, the glucose binds to the electrode resulting in a saturation of the electrode surface over time, rendering it unresponsive. The reaction is, however, principally reversible, and the reaction is pH dependent. Phenyl-boronic acid derivatives can be tailored for the necessary binding strength and specificity, opening up many possibilities for further research towards continuous glucose monitoring with these stabilizing agents combined with new glucose detection technologies.[15]

Artificial enzymes

Early research used pure metal electrodes, usually Pt, to oxidize glucose. The glucose concentration is amperometrically determined by oxidizing the glucose molecule, and measuring the resulting current. However, glucose is chemically very stable, resulting in poor detection accuracy. More recent research has demonstrated that by varying electrode composition and (nano) structure, glucose oxidation can be catalysed and selectivity can

be enhanced, thereby improving charge transfer efficiency.[3,7,49,68] In electrochemical measurements, increasing the surface area of the electrode increases the number of glucose molecules for detection, and therefore the detection signal. The most straightforward method to achieve this is to use roughened or nanostructured electrodes. A considerable range of materials has been investigated in this context, and the results are discussed in a comprehensive review by Toghill and Compton in 2010,[7] and in a more recent review of nanostructured electrodes by Zaidi and Shin in 2016.[68] In addition, there is a review on metal nanostructures as artificial enzymes by Tee *et al.* (2016) which focusses on the linear detection range and the LOD.[82] We will briefly highlight some commonly used electrode materials and structures.

Copper oxide (CuO) is easily fabricated, low cost and has shown good potential as an electrode material for glucose detection. As a result CuO films and nanostructures have been widely investigated. Annealing CuO at high temperatures in order to remove all the water from the material, results in a highly porous, stable, nanostructured film.[83] It was shown that these structures can detect glucose concentrations between 0.40 μM to 2.00 mM. CuO nanoparticles deposited onto other substrate materials have been tested and also demonstrate high selectivity for glucose.[68] Other structures include CuO nanowires on Cu and CuO nanoflowers.[84,85]

Gold (Au) is stable, biocompatible and conductive, making it a popular electrode material. Gold nanostructures are easily synthesised, and in the last decade Au nanoparticles were shown to be very effective for glucose oxidation.[7]

Electrodes of combined metals have been shown to have improved electrocatalytic activity in comparison to single metal electrodes due to functionalization of the surface.[68] Au nanoparticles deposited on NiO proved to work well, in addition, positive results came from Pt–Pd on Au (LOD of 20.6 μM)[86] or graphene.[87]

Co_8S_9 has been used as an artificial enzyme.[88] It has been reported to detect both glucose and hydrogen peroxide with an LOD of 0.45 μM, and a linear response between 21.50 μM and 1.18 mM.

Co_3O_4 doped with Sn has had promising results.[89] Sn improves the conductivity of Co_3O_4 significantly. The linear response was reported between 2 μM and 0.5 mM; and 0.6–5.5 mM, with an LOD of 100 nM.

Nickel hydroxide/3D graphene electrodes with an LOD of 24 nM[90] were reported in combination with electrochemical measurements.

Frequency-resolved spectroscopy

Direct current (DC) measurements can be applied to detect glucose concentrations *via* electron transfer between the glucose molecule and the electrode (glucose oxidation) as discussed above. Alternatively, variations in glucose concentration will impact the conductivity of the bodily fluid. However DC conductivity measurements on body fluids, particularly sweat, will further be strongly influenced by temperature, pH as well as other biomarkers and salts. For more accurate glucose detection, frequency-resolved techniques, such as impedance, admittance, dielectric and terahertz (THz) spectroscopies, offer more insight.

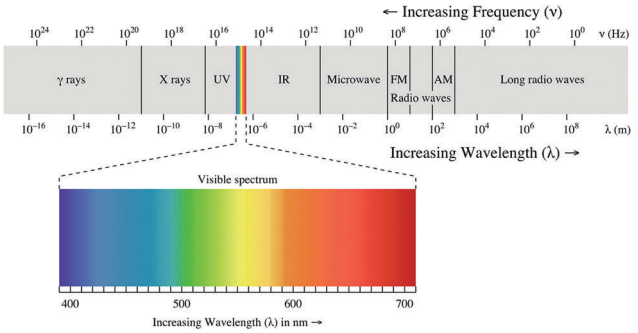

Fig. 6 The electromagnetic spectrum. The specific frequencies ranges that are relevant for different optical spectroscopic techniques are labelled. Image from Wikimedia, original by Philip Ronan.[91]

These techniques monitor the response of the sample to a modulated electric field. The electric field is modulated over a range of set frequencies during the measurement, resulting in a frequency spectrum with information about relaxation processes. The difference between the techniques is the frequency range under investigation. Fig. 6 illustrates the frequencies in the electromagnetic spectrum.[91] Impedance and admittance spectroscopy are electrical measurements and are used to study frequency windows between sub Hertz (Hz) and 10^6 Hz. The results yield information about the resistive/conductive and capacitive response of the sample. Dielectric spectroscopy is a non-contact method used to study the frequency response of a sample at around 10^7–10^9 Hz, and typically yields information about structural properties and intermolecular interactions. THz spectroscopy is an optical spectroscopy that probes the frequency response of a sample in the THz regime, *i.e.* at 10^{12} Hz. This enables the investigation of even faster relaxation processes and interactions than with impedance or dielectric spectroscopy.

The glucose molecule exhibits several relaxation processes at characteristic frequencies, which can be related to distinct physical processes. At low frequencies (sub Hz – 10^3 Hz) the translational motion of the glucose molecule in solution, *i.e.* DC conductivity, dominates the frequency spectrum. At higher frequencies (10^3–10^7 Hz) a relaxation related to cooperative interactions between the glucose molecules is observed. Further relaxation processes at intermediate frequencies have been observed for cooled samples with higher glucose concentrations.[92] Their origin is under debate, but they have been linked to clustering of glucose molecules.[93] These processes are most likely irrelevant for glucose detection under physiological conditions. THz spectroscopy has been used to probe characteristic glucose–water interactions.[94]

Skin impedance was measured by Caduff *et al.* (2009) in a non-invasive glucose monitor using impedance.[52] Measurement sensitivity can be increased by using electrodes with nanostructures[90] or by binding glucose to the electrode surface. The accuracy of impedance measurements for glucose detection were significantly enhanced with a pyrene-based boronic acid,[95] however the functionalized group bonds with glucose, making this specific derivative unsuitable for continuous glucose monitoring.[15]

Kim *et al.* (2015) made a reusable biosensor chip for measuring at radio frequencies.[96] The impedance, capacitance and other properties could be derived. These results (especially the impedance) showed linear responses to glucose concentrations. The measurements take 2 seconds and the chip can easily be flushed and reused. It showed a LOD of 0.033 μM.

The company gluco-wise published their *in vivo* results in Nature.[97] Their approach can measure dielectric permittivity through a finger or earlobe at radio wave frequencies. The set-up can successfully detect a glucose spike in humans, but the signal remains too uncertain for determining the exact concentration. The LOD was reported to be 0.4 mM.

Photo-acoustic spectroscopy is based on the principles that higher glucose concentrations result in higher acoustic pressure. A device sends ultrasonic waves through the tissue at high frequencies (20 kHz).[3,68,98] Acoustic spectroscopy works by radiating certain wavelengths (usually radiowaves) through a sample (or skin) and measuring the response.

Lee *et al.* (2015) demonstrated a sensor based on THz nano-antennae that could detect many analytes including glucose.[99] The sensor could detect differences in D-glucose concentrations varying over 3 orders of magnitude, from 10 to >4000 mg dL^{-1}.

Optical spectroscopy

The interaction of light with a molecule is very specific to its chemical structure, and can therefore be exploited for detection. Light incident on a sample may be absorbed, transmitted, reflected or scattered. For chemical identification, the detection of absorbed or scattered light is most relevant. The energy of the absorbed photon may promote an electron to a higher energy state, resulting in the subsequent emission of light, or the dissipation of heat. The benefit of using optical spectroscopy is that pH and temperature fluctuations are not critical for the measurement. Further, there is good flexibility in performing measurements, and optical signals are generally harmless to the skin.

There are, however, hurdles for applying optical spectroscopy in real applications for glucose detection. These are related to inhomogeneous samples, poor signal-to-noise due to low glucose concentrations, and the presence of other molecules with similar optical properties. Further, invasive calibration of the measurement is often necessary.[50] Considerable research into optical spectroscopy of different bodily fluids for glucose detection has been done, as well as optical measurements of the skin.[12] The following techniques can mostly also be used in combination with enzymatic substrates for a higher sensitivity.

Fluorescence spectroscopy

Fluorescence spectroscopy is used to monitor the emission of visible light from optically excited molecules. Fluorescent molecules are called fluorophores. Absorption of a photon promotes the fluorophore into the excited state, after which it returns to the ground state *via* emission of a photon with a characteristic energy. Glucose does not fluoresce, and must therefore be combined with a fluorophore for detection. The best fluorophores are conjugated molecules such as dyes, conducting polymers and carbon nanotubes. The fluorophore's emitted wavelength changes due to the interaction with glucose. *Via* the change in signal, the concentration of glucose can be detected.[77] Many variations of this approach have been tested, including stabilizing glucose anomers with boronic acid on a fluorophore.[3,6,14]

Vibrational spectroscopy

The most widely used vibrational spectroscopic method is Infra-Red (IR) spectroscopy.[22] Infrared spectroscopy (IR) is a chemical analysis method that investigates the absorption of infrared radiation in the wavelength range between 700 and 2500 nm by molecular vibrations. The wavelength absorbed by the molecule corresponds to energies of specific vibrations and rotations of bonds within the molecule. The challenges for glucose detection with IR are that the scattering is very high, glucose concentrations are too low for accurate detection, water yields a large peak that masks the glucose signal, and heterogeneous distributions of glucose can falsify the measurement.[100] Raman spectroscopy is an analogous technique to IR, but the physical background of Raman spectroscopy is often considered less intuitive than the physical background of IR spectroscopy. While IR probes the change in dipole moment associated by light absorption by a vibrational mode, Raman probes the change in polarizability associated with light interacting with a vibrational mode. The methods are complementary, and if a mode is Raman active it is not IR active and *vice versa*. A Raman measurement is performed by exciting the sample with a laser and detecting the Raman shifted (inelastic scattered) light. The Raman signals are weaker than IR signals, but Raman spectroscopy presents several advantages. Firstly, water does not give as prominent features in Raman spectra, IR is performed in transmission and requires substrates that are transparent in the IR wavelength range, while Raman spectroscopy measures scattered light and can therefore be performed on any surface, including opaque substrates. This enables the use of specialized substrates that enhance the Raman signal, *i.e.* Surface Enhanced Raman Spectroscopy (SERS).

Both vibrational spectroscopy methods can be applied to accurately identify and distinguish molecular species *via* the characteristic energy of molecular bonds. Further, the signals are stoichiometric; the signal intensity is directly proportional to the concentration of the analyte.

In 2006 Stuart *et al.* demonstrated *in vivo* Surface Enhanced Raman Spectroscopy (SERS) using silver film over nanospheres (AgFON).[41] Electrode nanostructures like gold nanostars have a lot of potential as electrodes for SERS, showing a LOD of 5 mM.[76] Recently organic layers were demonstrated as interesting SERS substrates. Yilmaz *et al.* (2017) synthesized a large thiophene-based molecule (DFH-4T) which enhanced the Raman signal by 3.4×10^4, and coated it with a thin gold layer to enhance the signal by 10^{10}.[101] Research is also focussed on electrode nanostructures or glucose-binding structures like albumin as well as different molecules based on boronic acid. Pandey *et al.* (2017) showed the effectiveness of chemometric algorithms to determine the concentration from Raman spectroscopy of the skin.[53]

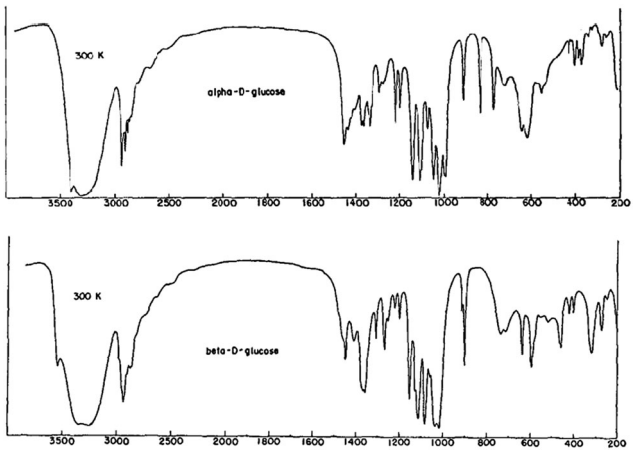

Fig. 7 Infrared spectra of D-glucose anomers. Top: α-D-Glucose spectrum. Bottom: β-D-Glucose spectrum. Reprinted from ref. 80 with permission from Elsevier.

The specificity of both Raman and infrared techniques is remarkable, and the α-glucose and β-glucose anomers yield clear differences in both spectra. The IR spectrum of the glucose anomers is shown in Fig. 7, with the α-glucose spectrum on the top and the β-glucose spectrum on the bottom. Specifically, the relative intensities of the peaks at 1480, 1360 differ, and two extra peaks for α-D-glucose appear between 750 cm^{-1} and 900 cm^{-1}. The peak at 1360 cm^{-1} represents the C–H bending at the anomeric centre. The peaks between 780, 840 and 915 cm^{-1} are all related to C–H deformation at the anomeric centre.[102] These three peaks at a similar energy can also be seen in the Raman spectra. The peak at 900 cm^{-1} is very clearly due to the β-D-glucose anomer as shown in detail in Fig. 8. The anomeric ratio can best be calculated from the peaks around 900 cm^{-1}.[29]

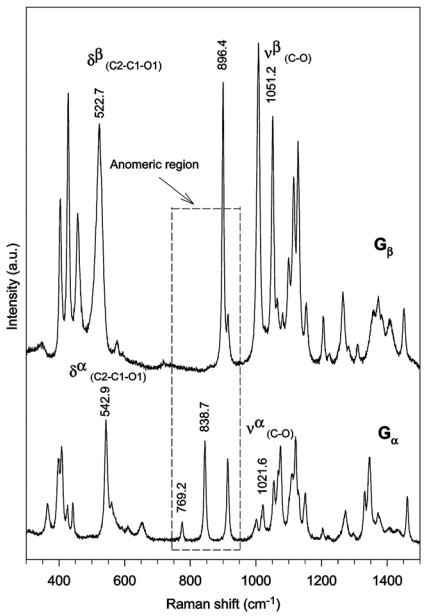

Fig. 8 Raman spectra of α-D-glucose and β-D-glucose centered at 900 cm^{-1} to show the anomeric region. Reprinted with permission from Dujardin *et al.* (2011).[29] Copyright (2011) American Chemical Society.

Autonomous insulin delivery system

An autonomous electrode-free glucose monitor was demonstrated by using synthetic polymer gel in combination with a phenyl-boronic acid derivative and insulin. The insulin-filled gel was tested on mice. It can be used as a synthetic pancreas as it releases insulin automatically when sugar levels rise. The gel lasts for 3 weeks before needing to be replaced.[15] They mention a LOD of less than 100 pM. The mechanism of releasing insulin is inventive. When there is a high concentration of glucose, the glucose binds to the boron polymer in the gel. The binding of glucose induces a hydration of the gel. This hydration induces the release of insulin from the gel, and it can subsequently diffuse into the bloodstream.

Improving accuracy in non-invasive glucose detection with hybrid approaches

Glucose detection based on a single technique is fundamentally limited by the sensitivity and accuracy of the technique itself. This is critical for quantifying low glucose concentrations in the presence of other molecular species. To circumvent this, it is possible to use a combination of complementary techniques for glucose detection, as well as sensors for monitoring the properties of the environment. To date, combining techniques for higher accuracy is not common practice.[103] Harman-Boehm *et al.* (2010) combined ultrasonic, electromagnetic and thermal sensors and averaged the results to obtain higher accuracy (96% of measurements are within the clinically accepted range). Song *et al.* (2014) combined IR and impedance for glucose monitoring.[71] The group used an Artificial Neural Network (ANN) for analysing the results. They had an MARD of 8.3%. Simulations of combining techniques was done by Asaduzzaman *et al.* (2016), they propose IR spectroscopy in combination with ultrasonic measurements.[98] The error was well within the required 20% range. Jintao *et al.* combined Raman and NIR spectroscopy and had a Relative Standard Deviation (RSD) of below 4%.[72] Similarly, Amaral *et al.* (2017) combined IR with impedance.[70] Caduff *et al.*, (2009) combined photoelectric spectroscopy and dielectric measurements to monitor glucose in skin and underlying tissue, with an MARD of 27%.[52] Generally combining multiple techniques presents a cost issue, as well as increasing the size and complexity of the sensor. However, it may be a promising strategy if the accuracy and precision can be improved beyond those of conventional glucose sensors.

The current market

Until 2010 the test strips and glucose meter was the only self-testing method available to diabetics. Though the number of test strip providers increased, four large companies dominate 90% of the test strip market (Abbott, Bayer, LifeScan, Roche), making market entry difficult for newcomers. Innovations in self-testing began to emerge in the late 00's, and since then the

Table 3 Overview of the current and emerging market of minimally invasive and non-invasive glucometers

Company/device	Technology	Comments	Accuracy (MARD)
Medtronics	Enzymatic detection in interstitial fluid	One week lifetime	9.1–13.6%
Ihealth Gluco Smart	Enzymatic detection in blood	Invasive	
DEXCOM G5	Enzymatic detection in interstitial fluid	Expensive weekly replacement is necessary; and a calibration every 12 hours.	9%
DEXCOM G6	Unknown... but no calibration is necessary. The other products from DEXCOM suggest that it is enzymatic.	Available in 2018	
Abbott's Freestyle Libre	Enzymatic detection in interstitial fluid	Expensive 2 weekly replacement is necessary	11.4%
Eversense Senseonics	Fluorescent polymer for interstitial fluid analysis	90 day lifetime Only approved in Europe Body insertion needs to be done by a doctor	11.4%
K-watch	Biosensing (enzymatic sensing) in interstitial fluid (*still in development*)		
Gluco-wise	Radio-wave frequency detection in blood (*still in development*)	Not accurate for specific concentrations	
NovioSense	Enzymatic detection in tears (*still in development*)	Considered non-invasive, but needs to be implanted in the eye	
Bigfoot	Glucose meter + automatic insulin pump (*still in development*)		
Tandem Animas Insulet	These companies will use DEXCOM's G5 meter in combination with their own smart devices (*all still in development*)		
Google – Novartis collaboration	Enzymatic detection *via* a circuit inside a soft contact lens. The data could be sent directly to a smartphone (*still in development*)		

market shifted towards continuous CGM devices.[104] Despite the massive demand and urgency for new innovations in glucose detection, in the last decades the enormous effort in research has only led to a few commercially successful products. This may be due to the fact that it is generally difficult for newcomers to enter the fiercely competitive medical market, with its lobby groups and administrative hurdles. In Europe, clinical trials are required in each independent country, which is expensive and time intensive. Further, the motivation to patent and develop new technologies quickly may mean that many researchers are not publishing their results and the developments in emerging technologies are not publically documented. Several larger companies have released continuous glucose meters (CGMs) based on enzymatic sensors, including Abbot, DEXCOM, and Medtronics.[13,17,50] Senseonics introduced Eversense on the market, a non-enzymatic chemical sensor.[13,105] Until 2010 CGM devices were not accurate enough to contribute to useful measuring and monitoring of patient glucose levels.[106] However, the most recent CGMs are more accurate and do improve patient control.[106] CGMs measure the glucose in blood or interstitial fluid using the commonly applied GOx or GDH enzyme combined with a calibrated glucose meter at very regular intervals (between 1 and 5 minutes, depending on the system). They require weekly or biweekly sensor replacements, with the exception of Eversense from Senseonics. Eversense applies a fluorescent glucose-selective polymer, and has an official lifetime of 90 days. Kropff *et al.* (2017), however, has shown 40% of sensors to be working after an impressive 180 days.[105] Regular calibration with blood samples is still recommended as these technologies are still being optimized. The systems are not always covered by insurance, and as a result, often expensive for patients. Freestyle Libre however, is insured in many countries and it is expected that other CGMs will follow worldwide. Several reviews on commercial CGMs exist; among others Lin *et al.* (2017) and Cappon *et al.* (2017).[13,17]

Non-enzymatic CGMs are not yet on the market for patient use, but some are already being tested on patients. Raman4Clinics, for example, is a platform for encouraging Raman spectroscopy as a medical analytical device. There are more non-invasive CGM systems emerging on the market.

Several products are currently being tested and are expected on the market in 2018. Two of these products are K'Watch (www.pkvitality.com) and Gluco Wise (www.gluco-wise.com). The K'Watch works with biosensors and nanoneedles which measure glucose in interstitial fluids. The biosensor needs to be replaced every month. GlucoWise uses low-frequency radio waves to measure the glucose in the blood. Neither of these monitor continuously but they can measure the glucose concentration at the press of a button. These companies do not always give full disclosure of their technologies, because they want to secure their intellectual property and/or market position. Table 3 summarizes current and emerging technologies in CGM.

Summary and outlook

Research in the field of glucose detection is thriving, which is positive for the vast and growing number of diabetic patients. New results for enzymatic and non-enzymatic detection are promising for the development of CGM devices. Commercial CGMs are on the market, and the benefits of regular and

continuous monitoring has been recognized by patients, health care experts, and insurance companies. Most of the commercially available CGMs monitor glucose in interstitial fluid. Interstitial glucose levels may indeed be more relevant for preventing long term damage due to hyperglycaemia. However this is still an invasive measurement. In addition current CGMs are enzymatic systems and thus need regular sensor replacements. Enzymatic glucose sensors have very high sensitivity and selectivity, which is why most commercial sensors are in fact enzymatic. Enzymatic sensors are, however, critical for applications that rely on stability for long term usage.

Non-enzymatic systems for monitoring glucose, in contrast, demonstrate better stability, but more research is needed to overcome other issues such as interactions with other molecules, anomeric selectivity and miniaturizing the detection system. The choice of bodily fluid for non-invasive detection isn't a trivial one. The possible body fluids for non-invasive glucose detection, including tears, sweat, and saliva, all have lower glucose concentrations than blood, and measurement accuracy and precision are therefore the major challenge. Interstitial fluid seems the most practical for non-invasive CGMs because it is the fluid surrounding the nerve cells, and the location just below the skin, but there are problems to overcome, such as fluid extraction and varying pH, which affects the anomeric ratio of glucose. This means that fluctuations in interstitial pH need to be accounted for. Tear fluid and sweat also have many advantages, but present equally difficult challenges.

Breakthroughs in non-invasive glucose detection require interdisciplinary efforts to fully understand the challenges, and required innovations to overcome current technical limits in glucose detection for self-monitoring devices. Future research will need to focus on both fundamental as well as applied questions, such as electrode structures and geometries, device miniaturization, glucose-binding surfaces and molecules, as well as (theoretical) insight into the mechanism of glucose mutarotation, including the effect of pH and temperature, the effect of environment on the electrical and optical properties of glucose. Overcoming these challenges can enable the development of smart wearable devices for non-invasive CGM. This would improve the quality of life of hundreds of millions of people by improving measures for the prevention and treatment of diabetes.

Conflicts of interest

There are no conflicts to declare.

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